08/975,519 CURRENTLY PENDING CLAIMS

Claims 2-15, 20-30, 53-56 and 70-120 are currently pending.

2. The method of claim 20, further comprising purifying adenovirus from said cell lysate by a process that [includes] comprises one or more chromatography steps.

3. The method of claim 20, wherein the cells are perfused with a glucose containing media at a rate to provide a glucose concentration of between about 0.7 and 1.7 g/L.

4. The method of claim 20, wherein said exchanging buffer involves a diafiltration step.

5. The method of claim 20, wherein said adenovirus comprises an adenoviral vector encoding an exogenous gene construct.

6. The method of claim 5, wherein said gene construct is operatively linked to a promoter.

7. The method of claim 6, wherein said promoter is SV40 IE, RSV LTR, β-actin, CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.

8. The method of claim 20, wherein said adenovirus is a replication-incompetent adenovirus.

9. The method of claim 8, wherein the adenovirus is lacking at least a portion of the E1-region.

10. The method of claim 9, wherein the adenovirus is lacking at least a portion of the E1A and/or E1B region.

11. The method of claim 20, wherein said host cells are capable of complementing replication.

12. The method of claim 20, wherein said host cells are 293 cells.

13. The method of claim 5, wherein said exogenous gene construct encodes a therapeutic gene.

14. The method of claim 13, wherein said therapeutic gene encodes antisense *ras*, antisense *myc*, antisense *raf*, antisense *erb*, antisense *src*, antisense *fms*, antisense *jun*, antisense *trk*, antisense *ret*, antisense *gsp*, antisense *hst*, antisense *bcl* antisense *abl*, Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, zac1, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11 IL-12, GM-CSF G-CSF, thymidine kinase or p53.

15. The method of claim 14, wherein said therapeutic gene encodes p53.

20. A method for producing a [pharmaceutically acceptable] purified adenovirus composition comprising:

a) growing host cells in a media;

b) [perfusing] providing nutrients to said host cells by perfusion or through a fed-batch process;

c) infecting said host cells with an adenovirus;

d) lysing said host cells to provide a cell lysate comprising adenovirus, wherein said lysis is achieved through autolysis of infected cells; and

e) purifying adenovirus from said lysate to provide a [pharmaceutically acceptable] purified adenovirus composition.

22. The method of claim 2, wherein the chromatography step is [comprises essentially] a single chromatography step.

23. The method of claim 22, wherein said chromatography step is ion exchange chromatography.

24. The method of claim 23, wherein said ion exchange chromatography is anion exchange chromatography.

25. The method of claim 24, wherein said anion exchange chromatography utilizes DEAE, TMAE, QAE, or PEI.

26. The method of claim 24, wherein said anion exchange chr matography utilizes Toyopearl Super Q 650M, MonoQ, Source Q or Fractogel TMAE.

27. The method of claim 24, wherein said ion exchange chromatography is carried out at a pH range of between about 7.0 and about 10.0.

28. The method of claim 20, further comprising a concentration step employing membrane filtration.

29. The method of claim, 28, wherein said filtration is tangential flow filtration.

30. The method of claim, 28, wherein said filtration utilizes a 100 to 300K NMWC, regenerated cellulose, or polyether sulfone membrane.

53. The method of claim 20, wherein said media is a serum-free media and said host cells are capable of growing in serum-free media.

54. The method of claim 20, wherein said cells are grown as a cell suspension culture.

55. The method of claim 20, wherein said cells are grown as an anchorage-dependent culture.

56. The method of claim 53, wherein said host cells have been adapted [adaptation] for growth in serum-free media [comprises] by a sequential decrease in the fetal bovine serum content of the growth media.

70. A method for producing an adenovirus composition comprising:

a) growing host cells in a media comprising glucose;

b) [perfusing] providing nutrients to said cells by perfusion or a fed-batch process at a rate to provide a glucose concentration of less than 2.0 g/L;

c) infecting said host cells with an adenovirus; and

d) harvesting and lysing said host cells to produce a lysate comprising said adenovirus composition.

71. The method of claim 70, wherein the cells are [perfused] provided nutrients at a rate to provide a glucose concentration of [less] between about 0.7 and 1.7 g/L.

72. The method of claim 70, wherein said adenovirus comprises an adenoviral vector comprising an exogenous gene construct.

73. The method of claim 72, wherein said gene construct is operatively linked to a promoter.

74. The method of claim 73, wherein said promoter is SV40 IE, RSV LTR, β-actin, CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.

75. The method of claim 70, wherein said adenovirus is a replication-incompetent adenovirus.

76. The method of claim 75, wherein the adenovirus is lacking at least a portion of the E1-region.

77. The method of claim 76, wherein the adenovirus is lacking at least a portion of the E1A and/or E1B region.

78. The method of claim 70, wherein said host cells are capable of complementing replication.

79. The method of claim 70, wherein said host cells are 293 cells.

80. The method of claim 72, wherein said exogenous gene construct encodes a therapeutic gene.

81. The method of claim 80, wherein said therapeutic gene encodes antisense *ras*, antisense *myc*, antisense *raf*, antisense *erb*, antisense *src*, antisense *fms*, antisense *jun*, antisense *trk*, antisense *ret*, antisense *gsp*, antisense *hst*, antisense *bcl* antisense *abl*, Rb, CFTR, p16, p21, p27, p57, p73, C-CAM, APC, CTS-1, zac1, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11 IL-12, GM-CSF G-CSF, thymidine kinase or p53.

82. The method of claim 81, wherein said therapeutic gene encodes p53.

83. The method of claim 70, wherein said cells are harvested and lysed *ex situ* us hypotonic solution, hypertonic solution, freeze-thaw, sonication, impinging jet, microfluidiz or a detergent.

84. The method of claim 70, wherein said cells are harvested and lysed *in situ* usin hypotonic solution, hypertonic solution, or a detergent.

85. The method of claim 84, wherein said cells are lysed and harvested using detergent.

86. The method of claim 85, wherein said detergent is Thesit®, NP-40®, Tween-20®, Bri 58®, Triton X®-100 or octyl glucoside.

87. The method of claim 70, further comprising purifying adenovirus from said cell lysate.

88. The method of claim 70, wherein said media is subjected to diafiltration.

89. The method of claim 87, wherein said purifying involves treatment of the cell lysate with a nuclease.

90. The method of claim 89, wherein said nuclease is Benzonase® or Pulmozyme®.

91. The method of claim 87, wherein said purifying includes chromatography using one or more chromatography steps.

92. The method of claim 91, wherein said chromatography is [comprises essentially] a single chromatography step.

93. The method of claim 92, wherein said chromatography step involves ion exchange chromatography.

94. The method of claim 93, wherein said ion exchange chromatography is anion exchange chromatography.

95. The method of claim 94, wherein said anion exchange chromatography utilizes DEAE, TMAE, QAE, or PEI.

96. The method of claim 95, wherein said anion exchange chromatography utilizes Toyopearl Super Q 650M, MonoQ, Source Q or Fractogel TMAE.

97. The method of claim 94, wherein said ion exchange chromatography is carried out at a pH range of between about 7.0 and about 10.0.

98. The method of claim 70, further comprising a concentration step employing membrane filtration.

99. The method of claim 98, wherein said filtration is tangential flow filtration.

100. The method of claim 98, wherein said filtration utilizes a 100 to 300K NMWC, regenerated cellulose, or polyether sulfone membrane.

101. A method for preparing a [pharmaceutically acceptable] purified adenovirus composition comprising:

b) growing host cells;

b) providing nutrients to said host cells by perfusion or through a fed-batch process;

[b)]c) infecting said host cells with an adenovirus;

[c)]d) lysing said host cells using [a lysing technique other than freeze-thaw] a process that includes hypotonic solution, hypertonic solution, impinging jet, microfluidization, solid shear, detergent, liquid shear, high pressure extrusion, autolysis or sonication to produce a crude lysate composition comprising adenovirus; and

[d)]e) purifying adenovirus from said lysate by a process that includes one or more chromatography steps without the use of cesium chloride density gradient centrifugation, to provide a [pharmaceutically acceptable] purified adenovirus composition.

102. The method of claim 101, wherein the cells are lysed by solid shear, detergent lysis, liquid shear or sonication, to produce a cell lysate.

103. The method of claim 102, wherein the cells are lysed by detergent lysis.

104. The method of claim 103, wherein the cells are lysed by detergent Thesit®, NP-40®, Tween-20®, Brij-58®, Triton X-100® or octyl glucoside.

105. The method of claim 104, wherein said detergent is present in the lysis solution at a concentration of about 1% (w/v).

106. The method of claim 101, wherein the cells are perfused in a glucose containing media at a rate to provide a glucose concentration of between about 0.7 and 1.7 g/L.

107. The method of claim 101, wherein said lysate is subjected to a diafiltration step.

108. The method of claim 101, wherein the chromatography is [comprises essentially] a single chromatography step.

109. The method of claim 108, wherein said chromatography step involves ion exchange chromatography.

110. A method for preparing a [pharmaceutically acceptable] purified adenovirus composition comprising:

a) growing host cells in a media;

b) infecting said host cells with an adenovirus; and

c) harvesting and lysing said host cells to provide a lysate comprising adenovirus; and

d) purifying adenovirus from said lysate by a process that includes a chromatography step without the use of cesium chloride density gradient centrifugation, wherein said chromatography step involves [essentially] a single chromatography step, to provide a [pharmaceutically acceptable] purified adenovirus composition wherein the recovery of purified adenovirus from the lysate after the chromatography step is 70% ± 10% of the starting PFU.

111. The method of claim 110, wherein said chromatography step involves ion exchange chromatography.

112. The method of claim 111, wherein said ion exchange chromatography is anion exchange chromatography.

113. The method of claim 112, wherein said anion exchange chromatography utilizes DEAE, TMAE, QAE, or PEI.

114. The method of claim 112, wherein said anion exchange chromatography utilizes Toyopearl Super Q 650M, MonoQ, Source Q or Fractogel TMAE.

115. The method of claim 110, wherein the adenovirus is subjected to purification steps that include reducing the concentration of contaminating nucleic acid.

116. The method of claim 110, further defined as comprising the steps of concentrating said crude cell lysate, exchanging buffer of said crude cell lysate, and reducing the concentration of contaminating nucleic acids in said crude cell lysate.

117. The method of claim 110, wherein said media is serum-free media and the cells are capable of growing in serum-free media.

118. A method for preparing a [pharmaceutically acceptable] purified adenovirus composition comprising:

a) growing host cells;

b) [perfusing said] providing nutrients to said host cells by perfusion or through a fed-batch process;

c) infecting said host cells with an adenovirus;

d) lysing said host cells using [a lysing technique other than freeze-thaw] a process that includes hypotonic solution, hypertonic solution, impinging jet, microfluidization, solid shear, detergent, liquid shear, high pressure extrusion,

autolysis or sonication to produce a crude lysate composition comprising adenovirus; and

e) purifying adenovirus from said lysate by a process that includes a chromatography step without the use of cesium chloride density gradient centrifugation, wherein said chromatography step involves [essentially] a single chromatography step, to provide a [pharmaceutically acceptable] purified adenovirus composition.

119. The method of claim 20, 70 or 118, wherein the [perfusion is achieved] nutrients are provided by a fed-batch process.

120. The method of claim 20, 70 or 118, wherein the [perfusion is achieved] nutrients are provided by [continuous] perfusion.